APPENDIX "A"

The affidavit of Dr. William Jia, pursuant to 37 C.F.R. 1.132, is attached hereto.

P444 0001
GOSO/GSL

Paper No.:____

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Inventor(s): HUANG, Dong; QI, Dong Feng

Title: NOVEL AGLYCON DAMMARANE SAPOGENINS, THEIR USE AS ANTI-CANCER AGENTS, AND A PROCESS FOR PRODUCING SAME

Serial No.: 09/910887

Filed: 24 July 2001

Examiner: Sabiha Naim Qazi Art Unit: 1616

Date: 21 July 2003

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

AFFIDAVIT UNDER RULE 1.132

I, William Jia, of 779 W.53rd Avenue, Vancouver, British Columbia, Canada, MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the matters sworn to herein, except where the matters are stated to be based on information and belief, in which case I believe them to be true.

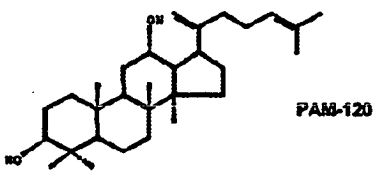
2. I obtained a Ph.D. in cell biology from University of British Columbia.

3. I am an Associate Professor in the Department of Surgery, Faculty of Medicine at the University of British Columbia, in Vancouver, British Columbia, Canada. I have held this position since 1994.

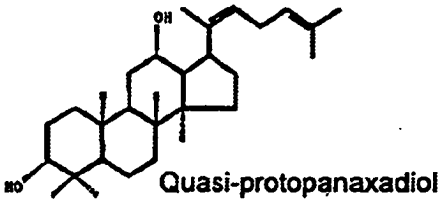
4. I am also the Chief Scientific Officer of PanaGin Pharmaceuticals, Inc., which is the assignee and owner of the rights in the invention which is the subject matter of United States patent application serial number 09/910,887 (the "Application").

5. I have read the Application, and I am familiar with the art to which it pertains.

6. I have supervised experiments which compare the efficacy of the compound dammara-20(21),24-diene-3,1β-diol (PAM-120), having the formula:



as an anti-cancer agent, against the efficacy of the compound dammara-20(22),24-diene-3β,12β-diol ("Quasi-protopanaxadiol"), disclosed in Japanese Patent Publication No. 08291194A ("Hideo"), having the formula:



7. I was unable to commercially obtain Quasi-protopanaxadiol. Therefore, in order to compare the efficacy of PAM-120 to Quasi-protopanaxadiol, I supervised the repetition of experiments described in a computerized translation of Hideo, which tested PAM-120 in the place of Quasi-protopanaxadiol. Attached hereto as Exhibit "A" is a computerized translation of Hideo obtained from the Japanese Patent Office website: www.jpo.go.jp.

8. The experiments were carried out under the conditions described in Hideo. Mouse leukemia cell line P388 and its multidrug resistant-cell line P388ADM were cultured ($1x10^6$ cells) in RPMI1640 culture medium supplemented with 20 μM mercaptoethanol and 10% fetal-calf-serum in a 5% CO_2 incubator. The cells were treated with either vinblastine or daunomycin at various concentrations (0.05-200 μg/ml for vinblastine and 1.55-2000 μg/ml for daunomycin, in two-fold series dilutions) for 48 hours, either in the absence of other drugs or in the presence of verapamil (at 6.25 μM) as a positive control, or PAM-120 (at 0.5 μM and 1 μM). The cells were also treated with PAM-120 alone at concentrations between 2.5 and 40 μM. After incubation, the numbers of viable cells remaining were measured with MTT assays. The IC50s were calculated by determining the concentrations of drugs required for 50% cell viability under various treatments. The results are shown in Table 1. The Resistance Factor, RF, between the multiple-drug resistant cells and the non-drug resistant cells was calculated as follows:

(RF) =IC50(P388ADM)/IC50 (P388).

Drugs	IC50 (ng/ml)*		RF
	P388ADM	P388	
PAM-120 Only	9.58 μM	6.66 μM	1.44
DAUNOMYCIN Only	507	6.11	83
DAUNOMYCIN + PAM-120 (0.5 μM)	3.19	3.18	1
DAUNOMYCIN + PAM-120 (1 μM)	0.885	1.78	0.497
DAUNOMYCIN + VERAPAMIL (6.25 μM)	51.7	5.41	9.56
VINBLASTINE Only	38.3	0.19	201
VINBLASTINE + PAM-120 (0.5 μM)	0.113	0.079	1.43
VINBLASTINE + PAM-120 (1 μM)	0.017	0.044	0.386
VINBLASTINE + VERAPAMIL (6.25 μM)	2.78	0.2	13.9

Table 1: Effect of PAM-120 on the Resistance Factor of P388ADM and P388 Cells. *The IC50s in the table are measured for either daunomycin or vinblastine in ng/ml, except as indicated for the measurement of the IC50 of PAM-120, which was measured in μM.

9. Although the multiple-drug resistant P388ADM cells are 83 times more resistant to

daunomycin and 201 times more resistant to vinblastine than the non-drug resistant P388 cells, the P388ADM cells are only 1.43 times more resistant to PAM-120 than the P388 cells.

10. By treating the P388ADM cells with daunomycin in the presence of 0.5 μM PAM-120, the daunomycin IC50 of the P388ADM cells is reduced from 507 ng/ml to 3.19 ng/ml, which is nearly the same as the daunomycin IC50 of the non-drug resistant P388 cells. Therefore, in the presence of 0.5 μM PAM-120, the P388ADM cells are equally as sensitive to the drugs as the non-drug resistant P388 cells are.

11. By treating the P388ADM cells with vinblastine in the presence of 0.5 μM PAM-120, the vinblastine IC50 of P388ADM is reduced from 38.3 ng/ml to 0.113 ng/ml.

12. The results in Table 1 demonstrate that PAM-120 can significantly sensitize the multiple-drug resistant P388 cells to chemotherapy agents. In the presence of 0.5 μM of PAM-120, the RF factor for daunomycin is reduced from 83 to 1, and the RF factor for vinblastine is reduced from 201 to 1.43. In the presence of 1.0 μM of PAM-120, the RF factors are reduced even further, to 0.497 for daunomycin, and 0.386 for vinblastine.

13. Using the same experimental conditions as described in Hideo, the IC50s of PAM-120 against cell lines P388 and P388ADM were determined to be 6.66 μM and 9.58 μM respectively. As disclosed in Table 1 in Hideo, the IC50 of Quasi-protopanaxadiol is 45.3 μM. (It is not disclosed which cell line this IC50 value was determined for, therefore, we have assumed that this is the IC50 value for the P388ADM cell line). Because PAM-120 has an IC50 concentration nearly 5 times less than the IC50 of Quasi-protopanaxadiol, PAM-120 is significantly more potent than Quasi-protopanaxadiol at inhibiting the cancer cell line. Furthermore, as shown in Table 2 below, PAM-120, at a concentration of only 1μM, is more effective than Quasi-protopanaxadiol, at a concentration of 37.5 μM, in sensitizing the multiple-drug resistant cell line P388ADM to daunomycin and vinblastine.

Sensitizer	Concentration Used (μM)	RF	
		Daunomycin	Vinblastine
Control (No sensitizer)	0.0	83	201
PAM-120	0.5	1	1.43
PAM-120	1.0	0.497	0.4
Quasi-protopanaxadiol	37.5 *	0.65	0.87

Table 2: Comparison of the Efficacy of PAM-120 and Quasi-protopanaxadiol on Sensitizing P388ADM cells to Daunomycin and Vinblastine (*Value obtained from Table 1 of Hideo).

14. Since the concentration of PAM-120 that enhances the efficacy of chemotherapy agents (0.5μM) is less than 1/10 of its IC50 (6.66μM) for the cells, PAM-120 may have a chemosensitizing effect on the cells. In contrast, the concentration of Quasi-protopanaxadiol (37.5 μM) needed to achieve similar enhancement in sensitivity is 83% of its IC50 (45.3 μM), suggesting part of the effect of Quasi-protopanaxadiol may be due to Quasi-protopanaxadiol's cytotoxicity on the cells, rather than a chemosensitizing effect. This indicates that there is possibly a difference in mechanisms of action between PAM-120 and Quasi-protopanaxadiol.

15. PAM-120 is significantly and unexpectedly more effective than Quasi-protopanaxadiol at inhibiting cancer cell lines P388 and P388ADM and sensitizing these cancer cell lines to chemotherapeutic agents.

SWORN before me at the)
City of Vancouver, in the)
Province of British Columbia,)
Canada this ~~21st~~ 14th day of ~~July~~ August)
2003.)
)
)
)
A Notary Public in and for the)
Province of British Columbia,)
Canada. My Commission is for life.)

William Jia

BC Drivers Lic #
DL: 5259222.

PERMANENT COMMISSION

Pauline Jang
Notary Public
305 - 1055 W Broadway

This is Exhibit "A"
to the Affidavit of
WILLIAM JIA
sworn before me on
this 14TH day of August 2003

Notary Public

Pauline Jang
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